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Acid Phosphatase Activity in Lymphocytes from Patients with *Spielmeyer-Vogt-Batten*'s Syndrome

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Summary: The purpose of the investigation presented was to study whether the lymphocytes from patients with *Spielmeyer-Vogt-Batten*'s syndrome deviate from normal with respect to acid phosphatase activity. The distribution of the activity seems to show that the patients with *Spielmeyer-Vogt-Batten*'s syndrome can be divided into two groups, viz. one in which the values are concentrated around the normal level, and another with increased values.

Aktivität der sauren Phosphatase in Lymphocyten von Patienten mit Spielmeyer-Vogt-Batten-Syndrom

Es wurde untersucht, ob die Lymphocyten von Patienten mit *Spielmeyer-Vogt-Batten*-Syndrom von denen von Normalpersonen bezüglich der sauren Phosphatase abweichen. Die Verteilung der Aktivität der sauren Phosphatase scheint zu zeigen, daß die Patienten mit *Spielmeyer-Vogt-Batten*-Syndrom in zwei Gruppen eingeteilt werden können: eine, bei der die Werte um den Normalbereich konzentriert sind und eine andere mit erhöhten Werten.

Introduction

The first case of juvenile amaurotic idiocy was reported by *Stengel* (1) in the Norwegian journal "EYR" in 1826. He described how all four children in a family successively became blind, imbecile and epileptic, and died at the age of about 20 years.

This clinical picture was later described by *Batten* (2), *Mayou* (3), *Spielmeyer* (4) and *Vogt* (5).

Spielmeyer-Vogt-Batten's syndrome is also called neuronal ceroid lipofuscinosis and is a progressive encephalopathy.

The first signs of the disease are seen after an apparently symptom-free period extending over several years, usually at the age of (4)-6-8-(10) years.

The predominant clinical feature is visual loss associated with pigmentary degeneration of the retina. Intellectual deterioration, extrapyramidal signs and spasticity are also common features, and epileptic seizures appear in the late stages of the disease.

Morphological changes in the white blood cells are of great significance. *von Bagh & Hortling* (6) drew attention to the occurrence of vacuolated lymphocytes. In a comprehensive study on 37 patients, *Rayner* (7) further showed that this trait behaves as a *Mendelian* dominant, being present in parents as well as in two thirds of the unaffected siblings.

Stubbe-Teglbjærg & Plum (8) were unable to find any relation between the number of vacuolated lymphocytes and the duration of the disease. On the other hand, they demonstrated wide variations in the number of vacuolated lymphocytes in the patients from week to week, without any relation to the clinical condition.

Many attempts have been made to elucidate the chemical structure of the vacuolated lymphocytes.

von Bagh & Hortling (6) studied vacuolated lymphocytes in smears stained by the *May-Grünwald-Giemsa* method. It is obvious that their preparations could not show any lipids although they may have been present. *Thiebaut* et al. (9) were unable to stain the vacuoles or their con-

tents. *Juliāo, Canelas & Long* (10) reported only negative findings both in stainings with Sudan Black and PAS. *Plum & Stubbe-Teglbjærg* (11) did not find a reaction to lipid stains in vacuolated lymphocytes, but some positive reactions for ribonucleic acid. *Kivalo & Stjernvall* (12) reported an electron-microscopic study of lymphocytes from patients with juvenile amaurotic idiocy. They found that the vacuoles seemed to consist of inclusions of very poorly absorbing fluid and gas. These findings were confirmed by *Plum & Stubbe-Teglbjærg* (13).

Spiegel-Adolf et al. (14) performed some histochemical studies on smears. Oil-red 0 and Sudan Black stains gave to a certain extent similar results. With both stains there was a number of vacuoles containing orange-red or black granules, but the majority of the vacuoles appeared empty.

Storti et al. (15) advanced the theory that the localization of the phosphatases in the cells is closely related to the lipid content and the amounts of polysaccharides and nucleic acid. As the metabolism of lipids, etc. is abnormal in *Spielmeyer-Vogt-Batten*'s syndrome, we studied the amount of acid phosphatase in the lymphocytes, which present an abnormal picture in this disease.

The purpose of the present work was to investigate whether lymphocytes from patients with *Spielmeyer-Vogt-Batten*'s syndrome deviate from normal with respect to acid phosphatase activity.

Using *Gomori*'s staining method (16) for the determination of acid phosphatase in tissues, *Rabinowich & Andreucci* (17) and *Storti* et al. (15) demonstrated the occurrence of acid phosphatase activity in bone-marrow cells, whereas they failed to reveal any activity in peripheral blood cells.

Other investigators (*Haight & Rossiter* (18), *Valentin & Beck* (15), *Rozenzajn* et al. (20)), who employed biochemical methods, were able to demonstrate the presence of acid phosphatase activity in cells both from the bone marrow and peripheral blood.

Finally, *Löffler & Berghoff* (21) demonstrated acid phosphatase activity both in bone-marrow and peripheral blood cells.

In the study reported below, two methods were used in the determination of acid phosphatase activity in lymphocytes, viz. (I) a purely histochemical examination of the cells in peripheral blood smears, and (II) isolation of the lymphocytes followed by chemical determination of the phosphatase activity.

Histochemical Determination of Acid Phosphatase in Granulocytes and Lymphocytes in Peripheral Blood Smears

Material and technique

We studied freshly prepared smears from 20 normal individuals (10 men and 10 women, hospital staff members aged 20–35 years), 15 hospitalized patients with epilepsy (9 men and 6 women aged 17–35 years, randomly selected) and 15 hospitalized patients with *Spielmeyer-Vogt-Batten*'s syndrome (7 men and 8 women aged 12–23 years).

All the patients with *Spielmeyer-Vogt-Batten*'s syndrome were admitted to Kolonien Filadelfia, where they had stayed for at least 12 months before the study The reason for their admission was that they could no longer remain in their homes or in other institutions because their disease was so far advanced that it was necessary to give them the care which can be provided only in a specialized hospital like ours.

The smears were stained by the method described by *Pearse* (8) as modified by *Rozenzajn* et al. (6).

The evaluation of the smears was graded on a 0–4 scale as follows:

Score	Result of staining
0	No stained granules
1	1–2 medium-sized or 1–5 small granules
2	2–4 large or 4–8 medium-sized granules
3	4–8 large or 8–16 medium-sized granules
4	Number of granules exceeding those for score 3

The scores thus obtained for 100 consecutive neutrophil granulocytes or 100 lymphocytes were then added and used in the determination.

Results

Table 1 and figure 1 show the values obtained. It is seen that wide interindividual variations in the activity, both in the neutrophil granulocytes and lymphocytes, were observed in the three groups.

Tab. 1. Acid phosphatase activity in neutrophil granulocytes and lymphocytes calculated on the basis of positive cells (see text).

	N	Mean	S.D.
Neutrophil granulocytes			
Controls	20	206	42
Epilepsy	15	193	47
Spielmeyer-Vogt-Batten's syndrome	15	199	60
Lymphocytes			
Controls	20	61	21
Epilepsy	15	73	31
Spielmeyer-Vogt-Batten's syndrome	15	89	24

N, number of individuals studied; S.D., standard deviation.

No difference in the activity of acid phosphatase in the granulocytes was demonstrated in the groups studied, whereas there was a statistically significant increase in the activity in the lymphocytes from the patients with *Spielmeyer-Vogt-Batten*'s syndrome as compared with the controls.

Chemical Determination of Acid Phosphatase Activity in Lymphocytes

Whereas the histochemical method (I) gives only a qualitative expression of the phosphatase activity in the cells, a quantitative determination of the activity can be performed by biochemical methods.

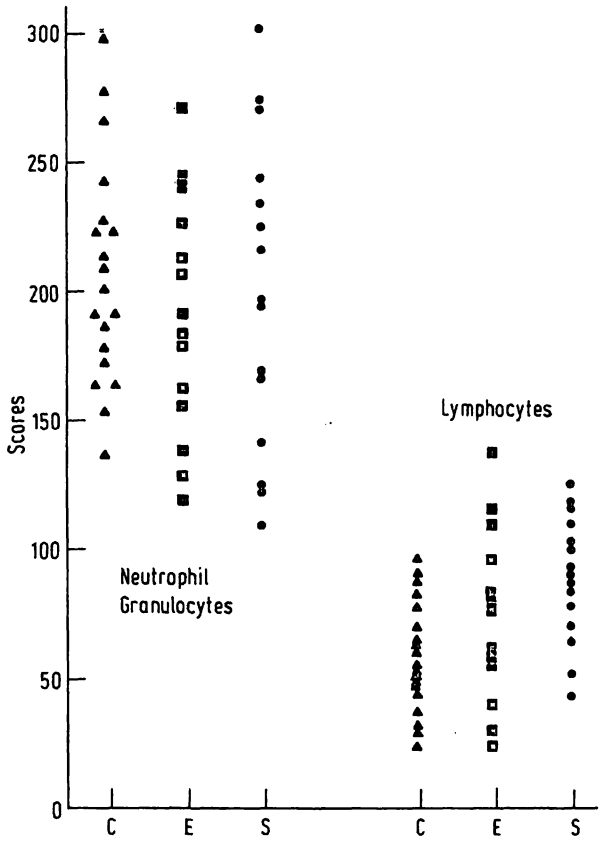

Fig. 1. Distribution of acid phosphatase in neutrophil granulocytes and lymphocytes.
Ordinate: Mean score for positive cells from each individual.
Abscissa: C, controls; E, epilepsy; S, *Spielmeyer-Vogt-Batten*'s syndrome.

Material and methods

In this part of the study, the control group consisted of 36 members of the hospital staff (18 men and 18 women), while the two study groups comprised 25 patients with epilepsy (15 men and 10 women) and all hospitalized patients with *Spielmeyer-Vogt-Batten*'s syndrome (4 men and 7 women). The latter group of patients were some of those studied by method I, while only a few of the patients with epilepsy were used in both parts of the study. Most of the subjects in the control group were the same in both parts of the study.

However, it should be noted that the samples used for methods I and II were not taken on the same day in the individuals studied.

The blood samples were taken in the morning after an overnight fast.

Preparation of lymphocyte suspensions

The separation of the cells, based on their specific gravity, was performed with the aid of "Ficoll-Paque" (Pharmacia Fine Chemicals, Sweden).

The phosphatase activity was determined by the method of *Bessey* et al. (22).

The amount of catalytic phosphatase (amount of enzyme) is expressed in international enzyme units (U), 1 U being equal to the amount which catalyses the hydrolysis of 1 μmol of *p*-nitrophenyl phosphate, corresponding to the formation of 1 μmol of *p*-nitrophenol per minute. The enzyme catalytic concentration is expressed in U/l at 37°C.

Results

The results are shown in table 2 and figure 2. It is seen that there was no distinct difference between the levels of acid phosphatase activity in the lymphocytes from the control group and the patients with epilepsy.

On the other hand, the group of patients with *Spielmeyer-Vogt-Batten*'s syndrome revealed an increase in the mean phosphatase activity, but the variations were so wide that the increase in the activity was not significant at the 5% level.

Tab. 2. Acid phosphatase activity (U) in lymphocytes calculated on the basis of 10^4 cells.

	N	Mean	S.D.	Minimum	Maximum
Controls	36	22.5	4.9	14.8	34.3
Epilepsy	25	22.8	3.9	16.2	31.4
Spielmeyer-Vogt-Batten's syndrome	11	30.3	11.2	18.3	48.3

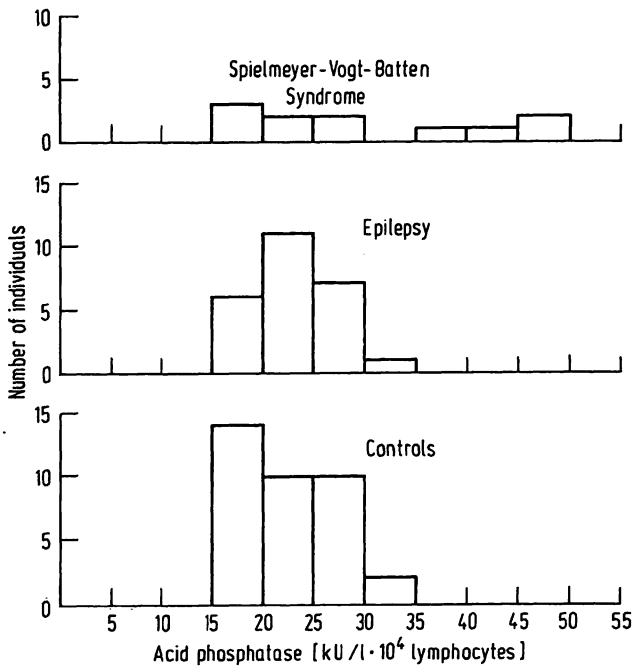

Fig. 2. Histograms showing the distribution of acid phosphatase in the three groups studied.

Conclusion

The studies of the acid phosphatase activity in the lymphocytes show that the mean activity is higher in the patients with *Spielmeyer-Vogt-Batten*'s syndrome, although it does not significantly deviate from normal.

The distribution of the activity seems to show that the patients with *Spielmeyer-Vogt-Batten*'s syndrome can be divided into two groups, viz. one in which the values are concentrated around the normal level, and another with

increased values. The values observed in the two groups seem to be independent of the duration of the disease. The group with increased activity included a pair of siblings.

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